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Review

At the right distance: ER-mitochondria juxtaposition in cell life and death☆

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ABSTRACT

The interface between mitochondria and the endoplasmic reticulum is emerging as a crucial hub for calcium signalling, apoptosis, autophagy and lipid biosynthesis, with far reaching implications in cell life and death and in the regulation of mitochondrial and endoplasmic reticulum function. Here we review our current knowledge on the structural and functional aspects of this interorganellar juxtaposition. This article is part of a Special Issue entitled: Calcium Signaling In Health and Disease. Guest Editors: Geert Bultynck, Jacques Haiech, Claus W. Heizmann, Joachim Krebs, and Marc Moreau.

1. Introduction

Incorporation of mitochondria, the prokaryotic endosymbiont, into the primordial eukaryotic cell was a turning point in evolution. Without the supply of energy provided by mitochondria, the evolution of higher organisms would not have been possible. These organelles not only provide energy but are also involved in important metabolic pathways, such as fatty acid oxidation, gluconeogenesis, steroidogenesis, synthesis of haeme and urea cycle [39]. In addition, mitochondria control apoptosis. Balance between pro- and anti-apoptotic proteins of the Bcl-2 family controls the release of cytochrome c, apoptosis-induction factor, Smac/DIABLO and endonuclease G from mitochondria. When retained in mitochondria, these proteins participate in cellular metabolism (e.g. cytochrome c shuttles electrons in the respiratory chain). When released to the cytoplasm, they function as proapoptotic factors, either by inducing apoptosome formation (cytochrome c) or by a direct enzymatic activity (endonuclease G). Finally, mitochondria cooperate with the endoplasmic reticulum (ER) not only to control calcium homeostasis but also to integrate signals of apoptosis. Here we review the molecular bases and the functional consequences of mitochondria-ER connection in cell life and death.

2. Mitochondrial morphology

At the ultrastructural level, mitochondria possess two membranes: the outer mitochondrial membrane (OMM) and the highly convoluted inner mitochondria membrane (IMM) [87,106]. The OMM is permeable to small peptides and metabolites due the presence of voltage-dependent anion channels (VDACs). The IMM is rich in the lipid cardiolipin and forms an intricate network of tubules and lamellae called cristae, where oxidative phosphorylation (OXPHOS) occurs. According to the chemiosmotic theory, the respiratory chain complexes pump protons across the proton-impermeable IMM, generating an

Abbreviations: ACAT1/SOAT1, acyl-coenzyme A:cholesterol acyltransferase-1; BAPTA-31, 75 kDa B-cell receptor-associated protein 31; BAK, Bcl-2 associated killer; BAX, Bcl-2 associated protein X; BCL-2, B-cell lymphoma 2; cholesterol, acyltransferase-1; Cho, cholesterol; DGAT2, diacylglycerol acyltransferase; Drp1, dynamin-related protein 1; ER, endoplasmic reticulum; ERMES, ER-mitochondria encounter structure; ERp44, Endoplasmic reticulum resident *protein* 44; FACL4, fatty acid CoA ligase 4 acyl-coenzyme A; Fis1, mitochondrial fission protein 1; GRP75, glucose-regulated protein; IMM, inner mitochondrial membrane; IP3, inositol 1,4,5-triphosphate; IP3Rs, inositol 1,4,5-trisphosphate receptors; RYRs, ryanodine receptors; IMS, intermembrane space; MAM, mitochondria associated ER membrane; MCU, mitochondria membrane uniporter; MiD49, mitochondrial division mitochondrial division 49; MiD51, mitochondrial division 51; Mff, mitochondrial fission factor; Mfn1, mitofusin1; Mfn2, mitofusin2; MITOL, mitochondrial ubiquitin ligase; OMM, outer mitochondrial membrane; OPA1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PA, phosphatidic acid; PACS2, phosphofurin Acidic Cluster Sorting Protein 2; PC, phosphatidylcholine; PDI, protein disulfide isomerase; PE, phosphatidylethanolamine; PEMT2, phosphatidylethanolamine methyltransferase 2; PML, promyelocytic leukemia protein; PS, phosphatidylserine; PS2, presenilin2; PTPIP51, protein tyrosine phosphatase-interacting protein 51; PTP, permeability transition pore; SAM, sorting and assembly machinery; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase pump; VDAC, voltage dependent anion channel; VAPB, vesicle-associated membrane protein associated protein B

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electrochemical gradient, whose free energy is used by the F1F0-ATPase synthase to phosphorylate ADP to ATP. Proton pumping is coupled to the transfer of electrons from NADH and FADH produced during the tricarboxylic acid cycle and other catabolic processes to molecular oxygen [82]. Both enzymes for intermediary metabolism and an autonomous circular small genome (mtDNA) encoding for proteins of the respiratory chain and for mitochondrial rRNA and tRNA are found in the matrix enclosed by the IMM.

Electron microscopy (EM) and live cell imaging allow us to appreciate the complexity of mitochondrial morphology. Electron tomography revealed that the IMM can be further subdivided in two different compartments, the inner boundary membrane and the cristae. The cristae are considered a separated compartment connected by narrow junctions to the inner boundary membrane. At the cristae level, the high density of respiratory chain component [43] assembled in supercomplexes increases OXPHOS efficiency. It is well established that the IMM is a dynamic structure able to rapidly change its shape in response to alterations in osmotic or metabolic conditions [56]. Pioneering studies performed by Hackenbrock in isolated mitochondria showed ultrastructural changes upon metabolic organelle activation. Mitochondria swifts from the so called orthodox to a condensed morphology characterized by a higher matrix electron-density and increased distance between IMM and OMM [56]. In addition to the dynamic changes in mitochondrial ultrasctructure, also mitochondrial morphology varies among different cell types: from spheres in hepatocytes to networks of elongated organelles in epithelial cells [26]. In myocytes, mitochondria are mostly globular in the perinuclear region; in the subsarcolemmal space, they are predominantly rod shaped whereas intermyofibrillar mitochondria have the same size of a sarcomere. Moreover, the different morphologies retrieved even in one single cell type highlight the requirement of a refined machinery that shapes the organelle, controlling constant organelle fusion and fission.

A systematic genome-wide screen for genes important for mitochondrial distribution and morphology performed in *Saccharomyces cerevisiae* revealed that not only core components of the fusion/fission machinery, but also that metabolic pathways, cytoskeletal proteins, factors regulating the interaction with other organelles all influence mitochondrial morphology [38]. We will now highlight the key molecules involved in fusion and fission of mammalian mitochondria.

3. Mitochondrial fission

Fission of mammalian mitochondria depends on dynamin-related protein 1 (DRP1), (DNM1 in yeast), as evidenced by the elongated mitochondria retrieved upon genetic inhibition of DRP1 [90,107]. DRP1 is a cytosolic protein whose mitochondrial localization depends on the interaction with OMM proteins. It was demonstrated that Drp1 functionally interacts with FIS1 [67,127]. Fis1 is not the sole DRP1 mitochondrial adaptor, since its downregulation does not abrogate mitochondrial translocation of DRP1 completely [72]. Moreover, FIS1 is involved in other processes: it induces ER dependent apoptosis and it participates in autophagy to remove damaged mitochondria [3,52,95,102]. Other partners driving the localization of DRP1 to mitochondria include mitochondrial fission factor (MFF) and mitochondrial division (MiD) 49 and 51. Ablation of Mff inhibits mitochondrial translocation of DRP1, impairs fission and causes mitochondrial elongation [47,85]. Interestingly, ablation of both FIS1 and MFF causes a phenotype less severe than DRP1 ablation knockdown or either MiD49 or MiD51 further increases mitochondrial connectivity [75]. Counterintuitively to their role as DRP1 receptors, MiDs overexpression causes mitochondrial elongation with increased mitochondrial recruitment of DRP1 [88,129]. The recruited DRP1 is however phosphorylated on S637 (a modification known to inhibit its activity), suggesting that this mitochondrial DRP1 has a dominant-negative effect that could explain the observed paradoxical elongation. It is tempting to speculate that MiDs regulate also localization of protein kinase A (PKA) on mitochondria, where it could phosphorylate DRP1 to trigger this autoinhibitory loop.

During different cell processes like apoptosis, neuronal and cardiac differentiation, DRP1 localization is regulated by its posttranscriptional modification. DRP1 translocation to mitochondria is controlled by phosphorylation and dephosphorylation especially upon mitochondrial dysfunction that is a key inducer of fragmentation. Dephosphorylation of DRP1 on SER637 (by calcineurin-cytoplasmic Ca^{2+} activation) induces its mitochondrial translocation and activation [18]. On the other hand, phosphorylation of this residue by PKA [28,20] and calmodulin-dependent protein kinase Ia (CAMK1a), [20] has opposite effects on mitochondrial morphology. While PKA causes mitochondrial elongation, CAMK1a causes mitochondrial fission through a yet unclear mechanism. Indeed, on the model DRP1 structure inferred from the crystal of the cognate Dynamin protein, phosphorylated Ser637 inhibits DRP1 oligomerization, an effect compatible with inhibition and not stimulation of fission. During mitosis phosphorylation of SER616 is driven by cyclin-dependent kinase 1 to control mitochondrial fission [117]. Mitochondrial DRP1 can then be stabilized on the surface of the organelle by SUMOylation [60,122], a process known to protect molecules from degradation by the ubiquitin–proteasome-system.

4. Mitochondrial fusion

Mitochondrial fusion is a two-step process where the outer and inner mitochondria fuse in separable event [76,108]. OMM fusion is performed by MFN1 and MFN2, two GTPases members of the dynamin family displaying high homology between them. Inner mitochondrial membrane fusion is mediated by Optic Atrophy 1 (OPA1). Both MFN1 and MFN2 localize in the OMM, anchored there by two TM domains. They display an effector GTPase domain and two coiled coil domain (CC1and CC2). The CC2 domains form antiparallel homo and heterodimers between MFN1 and MFN2 [21,71]. Mfns show differences in GTPase activity and GTP binding capacity. While MFN1 hydrolyses GTP faster than MFN2, the GTP binding capacity of the latter is higher [64]. Anyway, the ablation of either MFN1 or MFN2 results anyway in the inhibition of the fusion reaction [21,23,42]. Embryonic fibroblasts lacking MFN1 or MFN2 display however distinct types of fragmented mitochondria suggesting that MFN1 and MFN2 are not functionally redundant [21]. In cells lacking MFN1, mitochondria fail to bind, suggesting that MFN1 functions in mitochondrial tethering while MFN2 functions in a later process of the fusion reaction, consistent with a lower degree of mitochondrial fragmentation observed in $Mfn2^{-/-}$ cells as compared to the $Mfn1^{-/-}$ ones. Given its higher GTP hydrolysis capacity, MFN1 fully complements mitochondrial morphology in $Mfn1^{-/-}$ and $Mfn2^{-/-}$ cells. In contrast, MFN2 completely complement $Mfn2^{-/-}$ but only 25% of $Mfn1^{-/-}$ fusion activity. This diversity between MFN1 and MFN2 was further substantiated by the finding of a functional fusion axis between OPA1 and MFN1. In the absence of MFN2, the inner–outer membrane fusion machinery composed by OPA1 and MFN1 is still intact and can provide a low degree of fusion resulting in the few tubular mitochondria of $Mfn2^{-/-}$ MEFs [23], potentially explaining the morphological differences between $Mfn1^{-/-}$ and $Mfn2^{-/-}$ mitochondria.

Fusion of IMM is mediated by OPA1, a dynamin related GTPase retrieved in a soluble form or anchored to the IMM facing the intermembrane space [1,34]. In humans it undergoes alternative splicing of exon 4, 4b and 5b, generating 8 different isoforms [1]. OPA1 is the target of different proteases and posttranslational modifications [65,99]. The most accepted model of OPA1 processing involves three different protease: the m-AAA proteases paraplegin or AFG3L2 [41,65] and/or the i-AAA protease Yme1L [54,108] that generate the low molecular weight forms and the rhomboid protease Parl that releases soluble OPA1 into the IMS [24]. Functionally, OPA1 controls both mitochondrial fusion and cristae morphology [23,55,65,83,84]. Its downregulation causes vacuolization of cristae and widening of cristae junctions [44,83], as a result of the disruption of a high molecular weight complex comprising IMM and IMS forms of the protein [25,44].

5. Mitochondria and Ca^{2+} signaling

Mitochondria can take up and store calcium following a cytosolic calcium increase. The OMM, but not the IMM is permeable to calcium ions. The low affinity mitochondrial uniporter channel (MCU) located in the IMM takes up Ca^{2+} into the matrix using mitochondrial membrane potential as driving force: indeed, disruption of membrane potential by uncouplers prevents mitochondrial Ca^{2+} uptake [6]. Moreover, mitochondria are able to store high amount of Ca^{2+} as phosphates in the matrix: calcium phosphates precipitates can account for 25% of the total mitochondrial dry weight [73]. Mitochondria also have Ca^{2+} extrusion mechanism, highlighting that they are a dynamic Ca^{2+} store which can participate in signal modulation. The Ca^{2+} efflux from mitochondria is mediated by Na + (H+)/Ca^{2+} antiporters that use the ion gradients across the IMM [9]. The mitochondrially derived Ca^{2+} enters into the cytosol, from where it can be taken up by the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) (Fig. 1). This relationship between mitochondrial and ER Ca^{2+} will be addressed in further details below. It shall be noted that mitochondrial calcium uptake not only affects the modulation of cytosolic calcium transient, but it also activates key enzymes of the Krebs cycle to modulate mitochondrial ATP production. However, mitochondrial Ca^{2+} levels must be tightly controlled, since Ca^{2+} overload induces opening of an IMM high conductance channel, the permeability transition pore (PTP), with detrimental effects on cell functions and viability [92].

5.1. ATP production is modulated by Ca^{2+}

During physiological Ca^{2+} responses, mitochondrial Ca^{2+} regulates the activity of three dehydrogenases of the Krebs cycle. Ca^{2+} stimulates the activating dephosphorylation of pyruvate dehydrogenase [36]; and by direct binding to the complex, it stimulates the activity of α-ketoglutarate [36] and of isocitrate dehydrogenases [37]. These in vitro studies have been confirmed in vivo, where Ca^{2+} mediated stimulation of intramitochondrial metabolism was addresses by verifying an increase NAD(P)H [40] and FADH2 [58] autofluorescence, indicative of a stimulation of reducing equivalents production. Finally a luciferase based probe for ATP indicated that Ca^{2+} uptake indeed stimulates mitochondrial ATP production in intact cells challenged with an IP3receptor agonist [68].

5.2. Ca^{2+} and the mitochondrial permeability transition

The downside of mitochondrial Ca^{2+} uptake is the activation of the PTP, a large-conductance unselective channel of the IMM. The molecular nature of the PTP has been a matter of intense debate for many years (see [10] for a review). Recent electrophysiological evidence indicate that the PTP conductance is retrieved in ATPase dimers and it is modulated by the reversible association of mitochondrial cyclophilin D (CyPD), the receptor for the PTP inhibitor cyclosporine A, to the stalk of the ATPase. PTP is reported to be activated in different pathophysiological conditions including increases in matrix Ca^{2+} levels, oxidative stress, and accumulation of free fatty acids [7,9,103,109]. PTP opening results in the collapse of the mitochondrial electrochemical gradient and in swelling of the organelle, associated with OMM disruption and release of the proteins normally located in the IMS, including the activators of apoptosis such as cytochrome c. However, in vivo evidence of mitochondrial swelling during apoptosis is scarce (the integrity of the organelle has been long considered a sign of apoptotic cell death) raising question as to whether the PTP participated in cell death. It shall be noted however that transient PTP openings accompany the process of cristae remodeling, triggered by canonical apoptotic inducers of the Bcl-2 family, controlled by OPA1 and required to release the bulk of cytochrome c normally stored in the cristae [44,101]. Moreover, certain apoptotic stimuli appear not to require mitochondrial Ca^{2+} uptake and hence the Ca^{2+} dependent PTP; others, conversely, like oxidants,

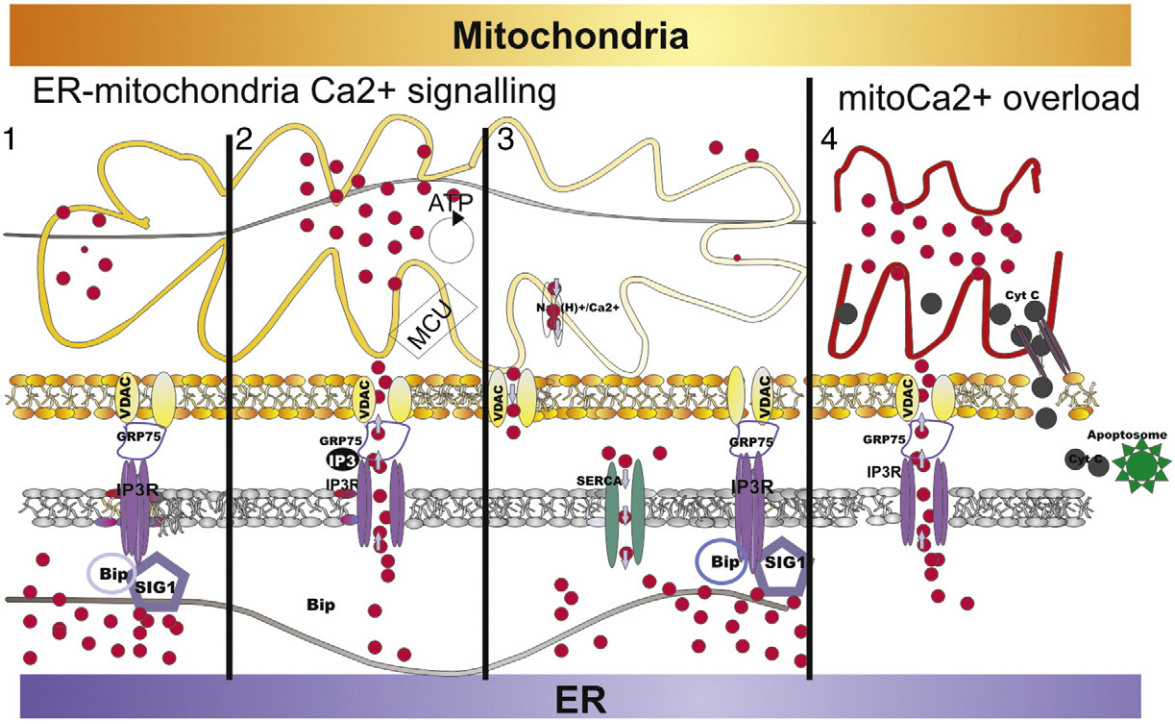

Fig. 1. The ER/mitochondrial Ca^{2+} shuttle. 1. During resting condition Ca^{2+} is stored within the ER lumen. 2. After IP3 stimulation Ca^{2+} is released into the cytoplasm by inositol 1,4,5-trisphosphate receptors (IP3Rs) and ryanodine receptors (RYRs) 3. Mitochondria take up the released Ca^{2+} that un turn activate three enzymes of tricarboxylic acid cycle (TCA cycle) increasing ATP production. Mitochondria later return the Ca^{2+} back to the cytosol by $Na^{+}(H^{+})/Ca^{2+}$ uniporter activity. Cytosolic Ca^{2+} is pumped back in the ER lumen by the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump. During normal signaling there is a flow of Ca^{2+} between these two organelles. 4. Mitochondrial Ca^{2+} overload induces the opening of the permeability transition pore (PTP), release of cytochrome c and apoptosis.

require adequate mitochondrial Ca^{2+} load [84]. Interestingly, apoptosis by these Ca^{2+} requiring stimuli is also controlled by the Bcl-2 family: for example, high levels of the antiapoptotic Bcl-2 reduce ER Ca^{2+} levels and mitochondrial Ca^{2+} uptake [73].

6. The interface between mitochondria and ER

As said above, mitochondrial Ca^{2+} uptake depends on the release of Ca^{2+} from the ER. We will describe this process in the next paragraphs. For now, it suffices to notice than in metazoan cells possessing the machinery for mitochondrial Ca^{2+} uptake, the interface between the two organelles must be organized to allow the generation of Ca^{2+} concentrations high enough to activate the mitochondrial Ca^{2+} uniporter (MCU). Otherwise mitochondria would be unable to take up calcium upon exposure to the low bulk cytosolic Ca^{2+} concentrations [92]. As reported below, the juxtaposition is retrieved also in yeast, where probably it serves other functions related to lipid metabolism.

The interaction between mitochondria and ER can be traced back to the unicellular eukaryote *Acanthamoeba castellanii*, a non-photosynthesizing free-living amoeboid protist (Amoebozoa) [124]. In Mammals, these regions of close interaction between ER and mitochondria are also known as MAMs (for Mitochondrial Associated ER Membranes), which are in reality a subdomain of the ER. Originally characterized from a biochemical point of view, MAMs display a particular sedimentation profile and composition that differ from the rest of the ER [119]. Key enzymes of lipid biosynthesis are enriched in this particular fraction, lending support to the idea that MAMs represent a particular ER subdomain. The first protein found in MAMs was Phosphatidylethanolamine N-methyltransferase 2 (PEMT2), an hepatic enzyme that converts PE to PC [30]. PEMT2 localizes exclusively in MAMs and EM images place PEMT2 in the proximity of mitochondria. Other proteins involved in lipid biosynthesis are enriched in this fraction, such as phosphatidylserine synthase [119], diacylglycerol acyltransferase (DGAT2) [110], acyl-CoA:cholesterol acyltransferase, and enzymes involved in the biosynthesis of the glycosylphosphatidylinositol anchors of proteins. This fraction is also enriched in Phosphatidylserine synthase (PSS) -1 and −2 [111], both enzimes catalyze catalyze serine exchange activity, whereas PSS1 catalyzes choline exchange [111]. DGAT2 localizes at MAMs and catalyses final step of triacylglycerol (TG) synthesis [110]. The most common protein used as MAM marker is Long chain acyl-CoA synthetase (ACS4) also known as FACL4 [74]. ACS4 catalyzes the initial step required for oxidation, elongation, and desaturation of fatty acids, for the synthesis of complex lipids and acylated proteins. Of the 5 isoforms of ACS, only ACS1 and ACS4 localize at MAMs, ACS1 being also found it at ER [74]. Another protein present in MAMs involved in lipid metabolism is acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1/SOAT1). Acat1 is involved in cholesterol ester formation and it is required for cholesterol availability in cell membranes [96].

In addition to the lipid metabolism proteins, proteins involve in Ca^{2+} homeostasis also reside in MAMs. For example, inositol triphosphate receptor (IP3R), the main ER Ca^{2+} channel, is enriched in MAMs from where it coimmunoprecipitates with the MOM voltage dependent anion channel (VDAC), physically associated to the chaperone glucose-regulated protein 75 kDa (GRP75) [115]. It is not clear whether IP3R has a role in the maintenance or regulation of physical contacts between ER and mitochondria since IP3R triple knockout cells have the same ER-mitochondria association than WT cell, suggesting the existence an IP3R independent linkage [29]. A summary of the key MAMs proteins is presented in Table 1 and Fig. 2.

The ER-mitochondria association is due to a presence of tethers that link both smooth and rough ER to the mitochondria. These tethers display a great diversity: tomography analysis of isolated liver mitochondria showed clusters of narrow particles connecting OMM to ER. The measured lengths of tethers between OMM and rough ER are 19–30 nm, between OMM and smooth ER are 9-16 nm [29]. Manipulation of the distance between ER and mitochondria using limited proteolysis demonstrated that ER and mitochondria are physically coupled by tethers of proteinaceous nature [29]. Limited proteolysis also decreased mitochondrial Ca^{2+} uptake, highlighting the importance of the ER-mitochondria physical interaction in Ca^{2+} transfer between the two organelles.

7. ER-mitochondria tethers in mammals and yeast

7.1. Yeast: the ERMES tether

A multiprotein complex responsible for mitochondria-ER tethering has been identified using a genomic screen in yeast. This complex called "ERMES" (ER–mitochondria encounter structure) is formed by Mdm10 and Mdm34, two integral OMM proteins; Mdm12, a cytosolic protein and Mmm1 (mitochondrial morphology maintenance 1), a protein located both in ER and OMM. ERMES appears as discrete puncta in region of close contact between ER and mitochondria. Remarkably, when a component of ERMES is missing, the complex falls apart and the puncta disappear, indicating that all the member of the complex are required for the mitochondria-ER association. Interestingly, deletion of ERMES complex components causes mitochondrial but not ER fragmentation. Strains lacking ERMES proteins display metabolic, phospholipid synthesis, maintenance of mitochondrial DNA and mitochondrial inheritance defects [13,63]. Several studies suggest a role of ERMES in mitochondrial DNA segregation. A member of ERMES complex, Mmm1, co-localizes with active replicating nucleoids [63] and immunoprecipitates with the nucleoid protein Mgm101 [77]. Another member of ERMES complex, Mdm10, is an integral OMM protein interacting with the sorting and assembly machinery (SAM) complex. The SAM complex assembles β-barrels in the OMM and in strains lacking Mdm10 the late step of mitochondrial outer membrane 40 (TOM40) assemblies is impaired, in turn hampering the formation of mature TOM complexes and mitochondrial protein import [78]. Further, ERMES is essential in key steps of lipid metabolism, promoting the transfer of ER-synthesized phosphatidylserine (PS) to mitochondria. Once in mitochondria, PS is converted to phosphatidylethanolamine (PE) by the phosphatidylserine decarboxylase (Psd1), an enzyme located in the inner membrane [121]. PE is converted into phosphatidilcholine (PC) in different reactions occurring at ER [70]. ER-mitochondria tethering disruption by deletion of ERMES component display a two- to fivefold reduction in PS-to-PC conversion rate despite Psd1 being active, proving the role of the complex in efficient inter-organellar phospholipid exchange. Whether ERMES is directly involved in lipid transfer between ER and mitochondria remains a matter of debate. Indeed, while direct measurements of PS to PE conversion indicated no differences in transport of PS from ER to mitochondria upon disruption of the ERMES complex, the steady state levels of mitochondrial PS were reduced and restored by the expression of an artificial tether.

Recently the Miro-like GTPase Gem1 was found to co-purify with the ERMES components Mmm1 and Mdm34 [70,112]. Gem1, like Miro, possesses two GTPase domain flanked by EF hands, and an OMM C-terminus anchor [70,112]. Mutation in the first GTPase domain abrogates ERMES localization. Even if strains deleted for Gem1 display less but larger ERMES foci, complex formation is retained, suggesting that Gem1 regulates ERMES organization [70]. Gem1 is synthetically lethal with enzymes of the cardiolipin and di-phosphatidylglycerol biosynthesis pathway, consistent with a role for Gem1 in regulating ERMES action on lipid metabolism. As said above, Gem1 belongs to the conserved class of Miro GTPases. Miro1&2 are involved in Ca^{2+} dependent mitochondrial movement mediated by the kinesin adapter Milton. Whether Miro display the same regulatory role on ER-mitochondrial connection in mammalian cells is unclear; in an interesting parallelism, however, Miro was reported to interact with Mfn2 [79] that as we will see below constitutes a physical ER-mitochondria tether in mammalian cells. Along the same line, ERMES components have been retrieved in non-fungal lineages, but no ERMES homologues have been so far identified

Table 1
Key MAMs component. For each protein component the evidence placing it at MAMs as well as the biological function (if known) in which it is involved are reported.

Protein	Subcellular localization	Methodology	Main function	Ref
PEMT2	MAMs	Percoll subfract/EM	Lipid metabolism	[30]
PSS2	MAMs	Percoll subfract/EM		[111]
DGAT2	MAMs	Percoll subfractionation		[110]
ACAT1/SOAT	MAMs/ER	Percoll subfractionation		
FACL4/ACS4	MAMs/peroxisome	Percoll subfractionation		
Calnexin	MAMs/ER	Percoll subfractionation	PACS2 regulates its MAM enrichment	[110]
IP3R	MAMs/ER	Percoll subfractionation/EM	Physical tethering	[115]
GRP75	interact with IP3 at MAMs	IP		
VDAC	interact with IP3 at MAMs	IP		
PACS2	interact with Calnexin at MAMs	IP		[105]
VAPB	MAMs	Percoll subfractionation/confocal colocalization		[32]
PTPIP51	interact with VAPB at MAMs	Percoll subfractionation/confocal colocalization		
Mfn2	OMM/MAMs/ER	Percoll subfractionation/EM/ET/cosedimentation/ confocal colocalization		[31]
PERK	ER/MAMs	Percoll subfractionation/confocal colocalization		[120]
DRP1	OMM/MAMs/ER	Confocal colocalization	Mitochondrial fission decreased ER-mito tethering	[66]
Fis1	OMM/MAMs	IP		
BAP31	ER/MAMS			
Rab32	OMM/MAMS	Optipret subfractionation	Interacts with calnexin, phosphorylation of DRP1	[14]
BIP	MAMs/ER	Percoll subfractionation/confocal colocalization	Stabilizes IP3R3 at MAMs	[62]
Sig1R	Interact with BiP at MAMs	IP		
Mitol	OMM/MAMS	Percoll subfractionation/confocal colocalization/ cosedimentation assay	Modulates Mnf2-dependent ER-mito tethering	[113]
DJ1	OMM/MAMS	Percoll subfractionation/confocal colocalization	Increases ER-mitochondria tethering	[86]
Parkin	OMM/MAMS upon stimulation	Confocal colocalization		[16]
Pml	MAMs/ER	Percoll subfractionation		[51]
ATG15	MAMS/ER (fed)	Percoll subfractionation	Autophagy	[59]
ATG4	MAMS	Percoll subfractionation/confocal colocalization		
STX17	MAMS/ER (fed)	EM/percoll subfractionation/confocal colocalization		
DFCP1	MAMS	EM/percoll subfractionation/confocal colocalization		
ERO1α	MAMs/ER	Percoll subfractionation/confocal colocalization	Leaves MAMs under reducing condition	[50]

in metazoans besides Miro1&2. It is unclear whether a different structure replaced ERMES in metazoans or whether MFN2 that interacts with Miro represents the metazoan tether, as we will see below.

7.2. Mammals: Mitofusin 2 as a tether

MFN2 localizes in OMM, ER and in MAMs and it regulates both mitochondrial and ER morphology. Our work identified that MFN2 is enriched at the ER–mitochondria interface and it connects ER to mitochondria via direct interactions between ER localized MFN2 and MFN1 or MFN2 retrieved in the OMM. Ablation of MFN2 causes fragmentation of ER, increases the distance between ER and mitochondria and impairs mitochondrial Ca^{2+} uptake [31]. The role of MFN2 in tethering the two organelles was confirmed in different systems: its ablation impairs lipid transfer between ER and mitochondria [4,57,123] and its conditional deletion in cardiomyocytes separates mitochondria from the sarcoplasmic reticulum and reduces mitochondrial calcium uptake [14]. The role of MFN2 as a tether was recently challenged in

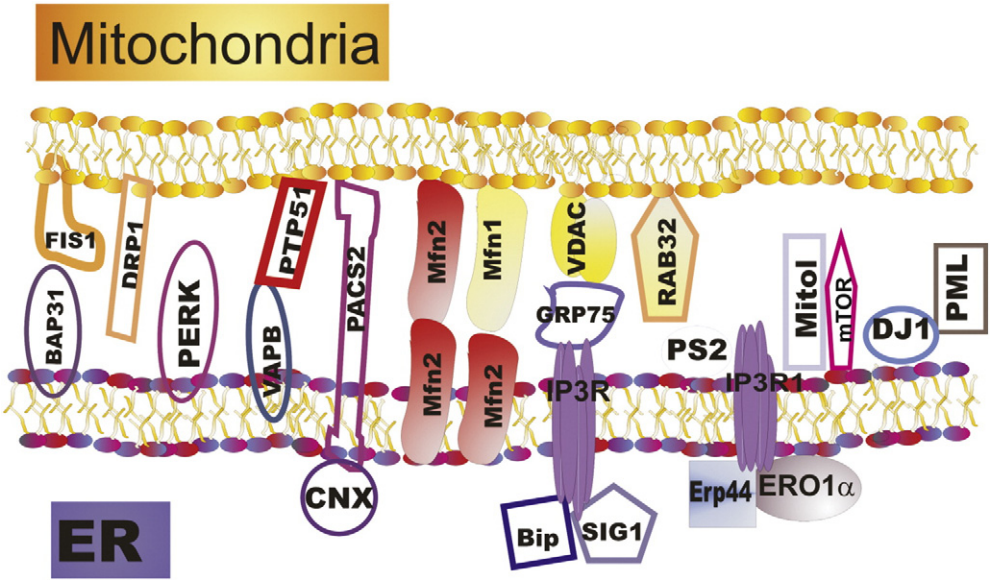

Fig. 2. A summary of proteins retrieved at the ER-mitochondria juxtaposition. Please refer to the text for the explanation of the acronyms and of the processes depicted here. ER-Mfn2 tethers ER-mitochondria by interaction with Mfn1 or Mfn2 located at mitochondria. VDAC at mitochondria and IP3Rs at ER are physically coupled trough the GRP75. Sig-1R forms complexes with the ER chaperone BIP. *Fis1* is physically associated with BAP-31. VAPB (vesicle-associated membrane protein associated protein B) at MAMs interacts with the outer mitochondrial membrane protein PTPIP51 (protein tyrosine phosphatase-interacting protein 51).

an electron microscopy study. Arbitrary definition of tethers as regions of ER-mitochondria distance of 10 or less nm and analysis of hundreds of such tether profiles led to the conclusion that in the absence of MFN2 tethers are actually increased. Based on this observation and contending that we identified the tether function of MFN2 only by using confocal microscopy, Cosson and colleagues conclude that Mfn2 is not a tether and explain our previous results as an artefact caused by the limited resolution of confocal microscopy [27]. While we acknowledge that Cosson et al. reproduced some of the aspects of our earlier work on MFN2, like the effect of Mfn2 ablation on ER shape, we believe that the conclusions by Cosson et al. on MFN2 and ER-mitochondria connection are technically and conceptually flawed for several reasons. First, the use of EM to visualize tethering is *per se* prone to artifacts due to the low sampling and to the arbitrary definition of tethers. How this approach is amenable to the investigator's interpretation is shown by another study that used the same EM approach to investigate the role of *Mfn2* ablation on mitochondria-sarcoplasmic reticulum tethering in the heart. This approach where the EM evaluation of juxtaposition contained a weaker bias (i.e., the tether distance was not arbitrarily defined and integral measurements of the contact surface between the two organelles was used as an indication of tethering) yielded the opposite result that *Mfn2* ablation decreases sarcoplasmic reticulum-mitochondria tethering [22]. Second, EM is a bi-dimensional technique whereas ER-mitochondrial tethers develop on three dimensions and therefore is prone to miss any tether that is not retrieved in the analyzed section. Indeed, electron tomography (the EM approach that inspects thick samples in all the three dimensions) analyses originally performed on cells lacking *Mfn2* demonstrated an increase in the mean distance between the organelles in the three planes [24]. Third, Cosson and colleagues wrongly assumed that the discovery of MFN2 as a tether was supported only by the confocal pseudocolocalization approach; however, electron tomography, cosedimentation experiments between genetically defined ER and mitochondria fractions, and cross-linking of ER and mitochondrial MFN2-containing complexes all concurred to conclude that MFN2 is a tether. Fourth, evidence from multiple laboratories pointed to a role for MFN2 in the functional aspects of ER-mitochondria exchange: its ablation lowers Ca^{2+} transfer in fibroblasts and in heart [22,113], and lipid transfer in a variety of cell types [5,57,59,123]. It shall be noted that a role for human Mfn2 in PS transfer from ER to mitochondria was recently challenged [69], but PS transfer was not followed over the full 24 hrs range required to complete the transfer. Moreover, *in vivo* studies on *Mfn2*$^{-/-}$ cells by Area-Gomez and colleagues [6] indicated that ablation of *Mfn2* resulted in a 25% reduction in mitochondrial PS transfer and PC synthesis, and in a 50% reduction in cholesterol transfer from ER to mitochondria. Finally, and perhaps conclusively, experiments performed by other groups using probes for ER-mitochondria tethering based on a split GFP that fluoresces only when the distance between the two organelles is below 20 nm (avoiding the potential issues of the pseudocolocalization approach) confirmed that the tethering is almost absent in *Mfn2* MEFs [2]. In conclusion, multiple lines of evidence support a role for MFN2 as a tether between ER and mitochondria and suggest that EM is not adequate to measure the distance between the two organelles.

The MFN2 containing complexes at the ER-mitochondria interface are large and likely comprise several other proteins. In a proteomic screening of MFN2 interacting proteins we retrieved trichoplein/mitostatin (TpMs), a mitochondrial putative tumor suppressor gene linked to intermediate filaments (IFs) [19]. TpMs is enriched in MAMs, and it negatively regulates ER–mitochondria tethering through its interaction with MFN2. By detaching the two organelles, TpMs protects against Ca^{2+}-dependent apoptotic stimuli. As noted above, MFN2 interacts also with Miro, the Gem1 orthologue in mammals. While this interaction has been involved in the explanation of the MFN2 -deficient mitochondria motility defects, the parallelism with the yeast ERMES complex seems very interesting. It is tempting to speculate that the tethers need to be linked to the organellar transport machinery. If this were the case, the ability of mammalian mitochondria to move in concert with the ER on acetylated microtubules [45] and the impairment in bud inheritance of ERMES-less yeast mitochondria would find a molecular explanation. In the case of mammalian mitochondria, altered co-transport of ER and mitochondria could represent a potential pathogenic mechanism for Charcot-Marie-Tooth IIa, where Mfn2 is mutated. If the disease associated mutations impair tethering, they might reduce the amount of ER transported to the axonal terminals together with mitochondria.

Another regulator of MFN2-dependent ER-mitochondria tethering has been identified in MITOL, a member of the membrane associated RING-CH E3 ubiquitin ligase family localized in OMM and MAMs. MITOL ubiquitinates K192 of MFN2 (absent in Mfn1) and the reduced ubiquitination of MFN2 retrieved in MITOL deficient cells correlates with reduced MFN2-dependent ER-mitochondria tethering and ER-mitochondria Ca^{2+} transfer [113]. Since defects in Mfn2 ubiquitination are a key event in familial Parkinson's disease, ER-mitochondria tethering might play an important role in the pathogenesis of Parkinson's associated neurodegeneration.

8. The function of the ER-mitochondria tethering

Of the mitochondrial surface, 20% is in close apposition with the ER [93]. It is therefore not surprising that many mitochondrial functions depend on this interface: Ca^{2+} uptake by mitochondria via the low affinity Ca^{2+} uniporter depends on the distance between the two organelles [91,93]. Alterations in this distance impact on mitochondrial metabolism [58] and the cellular response to Ca^{2+}-mediated cell death stimuli [102]. Disruption of ER-mitochondria contacts impacts on lipid metabolism [119]. Finally, the importance of this close juxtaposition is illustrated by its new role as a platform for autophagy, ER stress and mitochondrial fission [46].

8.1. Lipid Transfer

MAMs were initially described as an ER subdomain enriched in proteins involved in lipid metabolism. Mitochondria import from the ER essential lipids such as PS and PC. The enzymes necessary for the synthesis of these lipids and of triacylglycerol are located in both ER and mitochondria, indicating that interorganellar lipid traffic must exist [8,35]. PS is synthesized at ER by the MAMs enzymes PSS1 and PSS2, and then is transferred to mitochondria where phosphatidylserine decarboxylase (PSD) converts it to PE. PE synthesized at mitochondria goes back to the ER where phosphatidylethanolamine methyltransferase 2 (PEMT2), another MAMs resident protein, synthesizes PC. Enzymes involved in triacylglycerol synthesis, like fatty acid CoA ligase 4 (FACL4), which mediates the ligation of fatty acids to coenzyme A (CoA) and other cholesterol metabolite; acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1/SOAT1) that catalyzes the formation of cholesterol esters; and diacylglycerol acyltransferase are all retrieved in MAMs. Moreover, cholesterol is imported to mitochondria where it can be transformed into steroids by the inner membrane cytochrome P450, as the first and rate limiting step in steroidogenesis [118] (Fig. 3). Furthermore, MAMs are also involved in sphingolipid metabolism, since a sphingomyelinase located in MAMs produces ceramide that is transferred to mitochondria in order to be converted into sphingosine-1 phosphate and hexadecenal. In conclusion, the juxtaposition appears needed for effective transfer of different lipids between ER and mitochondria [125].

8.2. Mitochondrial fission

Recently, using three-dimensional (3D) EM and tomography images of ER-mitochondria interactions, tubular ER emerged as the marker sites of mitochondrial fission, before severing takes place [46]. Indeed, ER

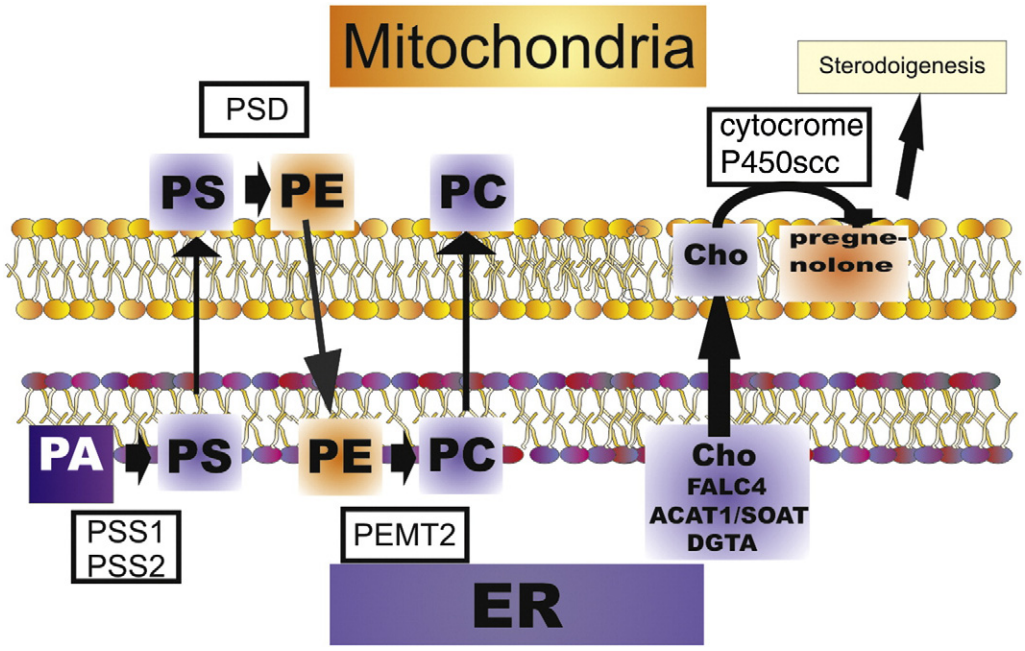

Fig. 3. The ER-mitochondria lipids transfer. PA (phosphatidic acid) is converted into PS (phosphatidylserine) by phosphatidylserine synthases 1 and 2 (PSS1, PSS2) in the ER. PS transfers to mitochondria where phosphatidylserine decarboxylase (PSD) converts it to phosphatidylethanolamine (PE). PE synthesized at mitochondria goes back to ER where phosphatidylethanolamine methyltransferase 2 (PEMT2) synthesizes PC (phosphatidylcholine). Enzymes involved in triacylglycerol synthesis also localizes in the MAMs, like fatty acid CoA ligase 4 (FACL4), which mediates the ligation of fatty acids to coenzyme A (CoA), acyl-coenzyme A:cholesterol acyltransferase-1 (ACAT1/SOAT1) that catalyzes the formation of cholesterol esters, and diacylglycerol acyltransferase. Cholesterol (Cho) is imported to mitochondria where it is transformed into pregnenolone by cytochrome P450scc in the first and rate-limiting step of steroidogenesis.

wrapping of mitochondria constriction precedes the recruitment of Drp1 by Mff. It is unknown which subdomain of the ER participates in mitochondrial fission: indeed, the known MAMs tethers in yeast and mammals did not affect the ER contacts with mitochondrial constriction sites [46], suggesting that another ER region could be involved in mitochondrial division. Moreover, Drp1 also displays an extra-mitochondrial localization: using immunofluorescense and EM experiments, Pitts et al. shown that Drp1 localize at ER [90]. Perhaps the original discovery of Drp1 at the ER is in reality a MAM localization however, this point can be only addressed using a combination of techniques including biochemical isolation of pure MAMs, mitochondria and ER membranes.

8.3. Autophagy

Autophagy is a degradative process for the breakdown and recycling of cellular components. It involves the formation of autophagosomes, multilamellar structure that engulf the components to be recycled, be either small cytosolic fraction or entire organelles, and then fuses with lysosomes, forming the autolysosome in which proteases degrade the cargo [17]. The origin of the autophagic membrane is quite clear in yeast, where it is called omegasome. Conversely, in mammals the point of origin is still under debate: different studies point to a mitochondria or an ER origin of the membranes [53]. Recently, the ER-mitochondrial contacts were described to be essential for autophagosome formation. Different proteins involved in autophagy were found to be enriched in MAMs after starvation-induced autophagy [59]: ATG14, ATG5 and DFC1 transfer and assemble in a MAMs complex. Interestingly, the disruption of ER-mitochondrial contacts by knocking down PACS2 or MFN2 decreases the number of autophagosomes, suggesting that MAMs integrity is a requirement for autophagasome formation. The impact of ER-mitochondria contact in autophagy is also evident when the autophagic membranes originate from mitochondria. According to this model, disruption of ER-mitochondria contacts by ablation of Mfn2 inhibit lipid transfer and starvation-induced autophagy, by inhibiting the PS transfer from ER into mitochondria-derived autophagosomes [57]. These studies highlight the importance of ER-mitochondria tethering in the progression of the starvation-induced autophagy. The MAMs serve as platform for autophagosome formation [59] and are indispensable for lipid transfer from ER to mitochondria and eventual autophagosome formation on the surface of mitochondria [57]. However, it remains unclear whether autophagosome originate only at MAMs and whether this localization is important only in starvation-induced autophagy. Future studies will shed new light on this novel function for the ER-mitochondria interaction sites.

8.4. Modulation of ER Stress

The ER plays crucial roles in protein synthesis, folding and sorting; it is also involved in phospholipid, cholesterol and steroid synthesis and, as previously mentioned, Ca^{2+} signaling. Accumulation of misfolded proteins, depletion of Ca^{2+}, protein synthesis impairment, blockage of ER-Golgi transport all result in a stereotypical rescue response called ER stress [126]. Severe or chronic decreases in the ER Ca^{2+} concentration diminish the proper function of the low affinity Ca^{2+} binding ER chaperones (calreticulin, BiP/GRP98 and GRP94), thus inducing ER stress [48]. ER stress triggers the activation of three ER membrane proteins (PERK; IRE-1α; ATF6) that induce the Unfolded Protein Response (UPR), which in its early stages favors cell survival (it increases ER chaperones, reduces other proteins translation and increases misfolded protein degradation), but eventually results in apoptosis [61] mediated by mitochondria [23]. It is therefore conceivable that the contact areas between ER and mitochondria participate in the regulation of ER stress and of the pro-death arm of it. Indeed, at least in a particular form of ER stress, it was shown that PERK localizes in MAMs and physically increases the contacts between ER and mitochondria. In $PERK^{-/-}$ cells mitochondrial Ca^{2+} uptake is decreased and mitochondria are protected from the damage generated by this type of ER stress [120]. Reciprocally, *Mfn2* ablation induces ER stress *in vitro* as well as in mouse and in *D. melanogaster* [81,83,61]. Ablation of *Mfn2* not only enhances the activity of the three branches of UPR response, but it also impairs

autophagy and apoptosis in response to ER stress. Moreover, MFN2 physically interacts with PERK to negatively regulate its activity [80]. The importance of this pathway is evident *in vivo*, where reduction of ER stress caused by *Mfn2* ablation in the mouse and in the fruit fly can correct the phenotypes observed in neurons, liver and skeletal muscle [33,100,104]. While ER stress could be a stereotypical response to mitochondrial dysfunction, it shall be noted that at least in the fruit fly ablation of the IM fusion protein Opa1 did not cause ER stress [33], pointing to a specific role for the ER-mitochondria connecting proteins in propagating the dysfunction across the interorganellar interface.

8.5. Generation of Ca^{2+} microdomains

Until the early 1990s, mitochondria were considered as bystanders in physiological Ca^{2+} signaling because of the biophysical properties of the mitochondrial Ca^{2+} uptake machinery. While the OMM VDAC is highly permeable to Ca^{2+}, the low affinity inner membrane mitochondrial Ca^{2+} uniporter (MCU) requires high Ca^{2+} concentrations that are not normally achieved in the bulk of the cytoplasm, where Ca^{2+} concentration is at rest 0.1 μM and upon IP3R or RYR stimulation it reaches 1–3 μM, well below the affinity of the MCU [97]. The general idea was that mitochondria could only take up Ca^{2+} when they were exposed to large Ca^{2+} loads like the ones observed during pathological events. However, this paradigm was challenged by Rizzuto and Pozzan, who by capitalizing on novel tools to directly measured the matrix Ca^{2+} concentration, observed mitochondrial Ca^{2+} uptake in living cells under physiological conditions [94]. To explain this discrepancy between the known low affinity of the Ca^{2+} uniporter and the response observed in cell led they proposed the so called "microdomains theory" according to which microdomains of high Ca^{2+} concentrations that largely exceed the ones recorded in the bulk of the cytosol exist at the interface between ER and mitochondria [91]. This theory was corroborated by the observation that both organelles are in close proximity in different cells types [93]; that release of Ca^{2+} from ER triggers mitochondrial Ca^{2+} uptake and activation of mitochondrial dehydrogenases [58]; and that it sustains mitochondrial ATP production [68]. The theory was directly demonstrated by using Ca^{2+} sensitive probes targeted to the OMM that indicated the generation of Ca^{2+} hot spots on the OMM after IP3-mediated ER Ca^{2+} release [49] and by the use of artificial ER-mitochondria linkers. The effect of the distance between mitochondria and ER in Ca^{2+} transfer was cleared demonstrated by Csordas et al. [29]. They generated a tether that decreased four times the distance between ER and mitochondria. Cells with enhanced ER-mitochondrial association after ER Ca^{2+} release displayed an increased mitochondrial Ca^{2+} uptake. Finally, mitochondrial Ca^{2+} uptake was reduced when the Mfn2 tether was genetically ablated [31], proving that the distance between the two organelles is a critical factor for mitochondrial Ca^{2+} uptake.

8.6. Regulation of cell death

Alterations of Ca^{2+} homeostasis that induce mitochondrial Ca^{2+} overload connect apoptosis to the ER-mitochondria interaction. The first evidence that ER-mitochondria Ca^{2+} cross talk participates in apoptosis came from Hajnóczky and coworkers, who demonstrated that IP3-induced and sustained Ca^{2+} mobilization from ER to mitochondria induces mitochondria Ca^{2+} overload, favoring PTP opening, cytochrome c release and apoptosis [116]. Conversely, the apoptotic response could be mitigated by reducing ER-mitochondria Ca^{2+} flux and thereby mitochondrial Ca^{2+} content. A reduction in ER-mitochondria Ca^{2+} flux can be achieved by decreasing steady–state ER Ca^{2+}, by inhibiting ER Ca^{2+} release or by increasing ER-mitochondria distance. The first strategy is employed by the antiapoptotic protein Bcl-2 which protects cell from Ca^{2+}-dependent apoptotic stimuli [89]. Similarly, knocking out the pro-apoptotic Bax and Bak proteins decreases ER Ca^{2+} content and renders cells resistant also to the apoptotic stimuli that require mitochondrial Ca^{2+} [102]. In order to reduce ER Ca^{2+} release, the easiest strategy is to decrease IP3R-mediated Ca^{2+} release. indeed, IP3R3 down-regulation or knock out blunts Ca^{2+} induced apoptosis [11]. The latter strategy of increasing the distance between ER and mitochondria is employed for example by Trichoplein/Mitostatin (TpMs), a putative tumor suppressor that negatively regulates Mfn2, reducing tethering and therefore apoptosis by Ca^{2+}-dependent stimuli [19]. Similarly, overexpression of NogoB, a member of the reticulon family that regulates tubular ER structure, increases the distance between ER and mitochondria and decreases ER-mitochondria phospholipid and Ca^{2+} transfer, as well as mitochondria-dependent apoptosis [114]. In keeping with this model, Ca^{2+}-mediated apoptosis is augmented upon: increased ER Ca^{2+} content, increased ER-mitochondrial tethering or sustained Ca^{2+} release from the IP3R. For example, upon apoptosis induction the ER-mitochondria distance decreases and the frequency of tight associations increases [29]. Similarly, sustained IP3R activation is a common feedback mechanism in apoptosis: in addition to activating caspase-3, released cytochrome c increases IP3R conductance, resulting in a sustained Ca^{2+} release that amplifies the apoptotic pathway [12].

There are several examples of conditions that impact on cell viability by engaging any of these mechanisms: for example, promyelocytic leukemia protein (PML) regulates cell survival trough ER-mitochondria Ca^{2+} signaling by modulating the phosphorylation state of IP3R [51]. A combination of increased ER-mitochondria tethering and sustained IP3R activation is observed during apoptosis in cells with different levels of the Alzheimer's disease associated protein Presinilin 2 (PS2). Its upregulation increases the physical interaction between ER and mitochondria and mitochondrial Ca^{2+} uptake after ER Ca^{2+} release [128]. Similarly, PS2 amplifies apoptosis: caspase 3 cleaves PS2 generating a presenilin-2-loop peptide (PS2-LP) which increases IP3R-mediate Ca^{2+} release [15]. Finally, also ganglioside accumulation in the glycosphingolipid-enriched microdomain of MAMs, where they interact with the phosphorylated IP3R form, promotes ER Ca^{2+} release, mitochondrial Ca^{2+} overload and apoptosis [98].

The MAMs resident proteins Rab32 and PACS2 affect apoptosis through a different mechanism that involves the modification of the pro-apoptotic proteins Bad and Bid. Rab 32 can function as a cAMP-dependent protein kinase (PKA)-anchoring protein (AKAP). Rab32 determines the association of PKA with MAM and mitochondria and thus modulates Bad phosphorylation on serine 155, thereby influencing the speed of apoptosis [14]. PACS-2 regulates the juxtaposition of ER-mitochondria through BAP31-dependent mitochondrial fission and after an apoptotic stimulus it induces mitochondrial translocation of Bid [105].

9. Conclusions

MAMs were initially characterized for their function in lipid metabolism. In the following years we understood that this interface is essential for mitochondrial Ca^{2+} uptake and Ca^{2+} signaling. We are now learning its importance in diverse cellular processes such as autophagy and ER stress. The number of proteins identified in MAMs is growing and it now comprises ER chaperones, ER Ca^{2+} channels and pumps as well as mitochondrial fusion-fission proteins. MAMs composition appears however dynamic, a feature well in accordance with the pleotropic function of this interface, with proteins that enter and exit the compartment depending on the patho-physiological conditions of the cell. For example, Ero1alpha MAMs localization depends on the redox cellular conditions [50] and proteins necessary for autophagosome formation translocate to MAMs when the cell is starved [59].

As we are starting to learn about the essential mediators of the juxtaposition process, we are also learning that ER-mitochondria distance can be modulated by cellular cues: in anorexigenic pro-opiomelanocortin neurons of the hypothalamus, diet-induced obesity decreases MFN2-dependent mitochondria-ER juxtaposition [100], whereas during

serum starvation tight ER-mitochondria contacts appear [29]. Apart from MFN2, the other physical tethers of mammals wait to be discovered and the mechanisms regulating tether formation and distance remain to be elucidated. Finally, altered function of ER-mitochondria contact sites has been implicated in Charcot-Marie-Tooth disease type II, Alzheimer's disease and Parkinson's disease, but whether it participates in pathological conditions other than neurodegeneration is unknown. We expect great discoveries on the composition, regulation, dynamics and involvement in disease of this amazing organelle interface.

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